The Laryngoscope

Hyaluronic Acid Fat Graft Myringoplasty: A Minimally Invasive Technique

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Objectives: Hyaluronic acid fat graft myringoplasty (HAFGM) is a new technique for tympanic membrane perforation (TMP) treatment. It is simple, inexpensive, and performed under local anesthesia at the outpatient office department. We aim to evaluate the HAFGM on different TMP sizes, to compare the success rate of HAFGM with the underlay and overlay techniques, and to assess the hearing improvement at one year postoperatively.

Study Design: Prospective study.

Methods: Patients were divided into three groups depending on the patient's choice of technique: HAFGM (group I), underlay technique (group II), and overlay technique (group III). Perforations were classified into four grades. Postoperatively, the status of the eardrum, the improvement of hearing, and the incidence of complications were the main criteria for measuring outcome.

Results: Distribution of TMP was 131, 63, and 52 in group I, II, and III, respectively. Global successful rate and successful closure of the grade I, II, III, and IV were the same for the three groups. Postoperatively, no worsening of bone conduction threshold was noted. Air-bone gap (ABG) was statistically similar for the three groups. No complications were noted for group I. The mean duration of the operative procedures was 16, 65, and 74 minutes for group I, II, and III, respectively ($P = .02$). The mean postoperative follow-up was 18.7, 20.6, and 15.5 months for groups I, II, and III, respectively.

Conclusions: HAFGM success rate is comparable to that of the underlay and overlay techniques. Furthermore, it requires no hospitalization and avoids the difficulty of overlay and underlay tympanoplasty.

Key Words: Tympanoplasty, myringoplasty, hyaluronic acid, fat graft, overlay, underlay, perforation, tympanic membrane, ear drum.

Level of Evidence: 1b

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INTRODUCTION

Longstanding tympanic membrane (TM) perforations may cause hearing loss and middle ear infection associated with an unpleasant discharge even if they are small in size. Furthermore, patients must avoid exposing their ears to water. Symptoms of tympanic membrane perforation include conductive hearing loss, aural fullness, and tinnitus.[1] Large varieties of tympanoplasty have been described in the modern literature, including use of a paper patch, gelfoam patch, autologous fat graft, in addition to the underlay and overlay techniques.[2] Tympanic membrane repair performed as an isolated surgical procedure (myringoplasty or type I tympanoplasty) or during a tympano-mastoidectomy yields variable success rates, with most of the reported scores higher than 80%.[1,3–5]

Fat graft myringoplasty (FGM) has been employed since 1962 for repairing small TM perforations.[6] The success rate of FGM for small perforations varies from 76% to 92%.[7–9] Failure rates for larger perforations are very high.[6–9]

Hyaluronic acid (HA) in its liquid form has been used in the middle ear without significant problems.[7] The solid polyester form is known to be fully reabsorbed within eight weeks.[10] In one study, Stenfors concluded that covering the defect of tympanic membrane with 1% hyaluronic acid repeated every second to third day has been shown to accelerate the closure of perforation size.[10] However, Prior et al. concluded that repair of TM perforations with hyaluronic acid ester films alone is not to be recommended because the success rate for his first five patients was 0%.[11] The study, however, was aborted at this point.

The association of HA to FGM known as hyaluronic acid fat graft myringoplasty (HAFGM) was reported in 2008, when the surgical technique was described and the results of this small series lacking a control group were presented.[12] In this study, we aim to evaluate the HAFGM on different TM perforation sizes, to compare the success rate of HAFGM to the underlay and overlay techniques, and to assess the hearing improvement at one year postoperatively. In addition, we discuss the uses and advantages of the HAFGM procedure in patients with longstanding dry TM perforations.

MATERIALS AND METHODS

Patients

This prospective study was conducted from 2007 to 2009 at the outpatient clinic of our otolaryngology department. All

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patients were adults and responded to the following inclusion criteria: 1) perforations present for at least six months, 2) without evidence of active chronic otitis media, cholesteatoma, or retraction pocket formation, 3) without suspected ossicular pathology on microscopic examination, and 4) air-bone gap (ABG) of 35 dB or better. Excluded were those with purulent discharge, suspected ossicular disease, suspected cholesteatoma, and unidentified anterior rim of the perforation. The size of the perforation was not considered an exclusion criterion. The investigation included 234 patients divided into three groups depending on the patient's choice of technique: HAFGM (group I), underlay technique (group II), and overlay technique (group III). All patients received a full description of the HAFGM procedure, as well as the underlay and overlay techniques. The temporalis fascia or the tragal perichondrium was the graft used for the underlay and overlay techniques. In the underlay technique, the graft was cut to size and inserted under the tympano-meatal flap onto the medial surface of the drum remnant. Gelfoam packing in the middle ear was used to support the graft firmly in position against the drum remnant. In the overlay technique, the graft was cut to size and placed over the perforation, lateral to the fibrous middle layer but under or medial to the squamous epithelial layer. To prevent graft lateralization, a small slit was made in the graft material and the graft slotted medial to the handle of the malleus when it was protruding.

Patients chose the technique most convenient to them and gave their informed consent.

Cases where the anterior rim of the perforation was not identified and hidden by the anterior wall bulging of the external auditory canal were excluded from the three groups to eliminate any bias because no drilling of bulging was done for the HAFGM group. Patients from group I were operated under local anesthesia in the office of the outpatient department.

Product Description and HAFGM Surgical Technique

Epidisc otologic lamina is a biomaterial composed of hyaluronic acid ester, a naturally occurring constituent of the extracellular matrix. The 8-mm-diameter transparent lamina has microperforations to allow permeability that facilitates drainage of exudates at the surgical site (Epidisc otologic lamina; Xomed-Medtronic, Jacksonville, FL). The HAFGM surgical technique was described in a previous study.[12] In brief, after the perforation's margins were de-epithelialized circumferentially, gelfoam pieces were placed into the middle ear through the perforation to support the fat graft. The fat graft was then inserted through the perforation as an hourglass-shaped plug. The lateral fat bulging should not be too high. Care should be taken to get an intimate contact between the epidisc, the fat graft and the tympanic membrane. The epidisc should carefully overlap, even if to a minimum extent, all the intact epithelium edge around the perforation. Depending on the TM perforation size, one or two HA epidiscs are placed over the fat graft. In the case of total perforation, the HA epidiscs cover the fat graft and the medial edge of the external auditory canal skin near the annulus. The HA is then covered with pieces of gelfoam soaked with ciprofloxacin, and the ear canal is filled with bacitracin/polymyxin ointment. Patients were discharged immediately after the procedure and instructed to keep their ears dry, to avoid plane traveling, and to prevent strong nose-blowing for two months.

Outcome Measures

The collected data of this study included gender, age, side, symptoms, and previous ear surgery in the affected side, the site of the perforation on the tympanic membrane, the size of the perforation, and the duration of surgery.

Ears were examined with an otomicroscope before the procedure, and the criteria of the perforations were documented. A photo-endoscopic image of each TM perforation was taken immediately before the procedure and at the second, fourth, sixth, and twelfth postoperative months. This allowed us to study the evolution of TM healing. Photos were taken by using a 4-mm-diameter, 6-cm-long, and 0° angulation endoscope (Karl Storz, Tuttlingen, Germany) connected to a Nikon Coolpix 4500 digital camera through a 590-70 connector (Karl Storz). The mean values of the pre- and postoperative air conduction (AC) and bone conduction (BC) thresholds at the frequencies 500 Hz, 1,000 Hz, 2,000 Hz, and 4,000 Hz served to calculate the air-bone gap closure. A postoperative increase of BC thresholds of 10 dB or more was considered a clinically significant sensorineural hearing loss.

Postoperatively, the status of the eardrum, improvement of hearing, and the incidence of complications were the main criteria for measuring outcome. Successful closure and graft failure rates were based on the status of tympanic membrane at the most recent visit, a minimum of 12 months postoperatively. A pinpoint perforation remaining postoperatively was considered a failure. Hearing improvement was assessed using the audiogram results obtained at 4 months and 12 months postoperatively. The first postoperative appointment was scheduled at 2 months or sooner if there was a complication. The follow-up was done at 4 to 6 months and 12 months after the procedure and then on a yearly basis.

TABLE I.
Gender, Side, and Age Repartition of Tympanic Membrane Perforation (TMP).

	Group I HAFGM	Group II Underlay	Group III Overlay
Number of TMP (n = 246)	131	63	52
Sex			
Male	45	30	25
Female	86	33	27
Side			
Right	70	29	31
Left	61	34	21
Age (years)	48 ± 18	26 ± 15	27 ± 18

Age is expressed in years ± standard deviation.
HAFGM = hyaluronic acid fat graft myringoplasty.

Statistical Analysis

A variance analysis with repeated measures and χ^2 tests was performed for statistical analysis. A $P < .05$ was considered statistically significant.

RESULTS

There were 234 patients included in our study. Only 12 patients had a bilateral perforation, all from group I. Distribution of TMP was 131, 63, and 52 patients in group I, II, and III, respectively. The mean age was 48 ± 18 (standard deviation [SD]) years in group I, 26 ± 15 (SD) years in group II, and 27 ± 18 (SD) years in group III. Age difference was statistically significant between groups I and II ($P < .0001$) and between groups I and III ($P < .0001$). Gender and side of perforations are represented in Table I.

TABLE II.
Size and Site of Tympanic Membrane Perforation (TMP).

Perforation		Group I HAFGM	Group II Underlay	Group III Overlay
Size	Small	41	32	16
	Medium	46	18	21
	Large	32	5	8
	Total	12	8	7
Site	Postero-superior	62	24	20
	Postero-inferior	90	37	33
	Antero-inferior	72	27	37
	Antero-superior	41	19	13

One patient may have a TMP affecting different sites.
HAFGM = hyaluronic acid fat graft myringoplasty.

TABLE III.
Success Rate Described by Tympanic Membrane Perforation (TMP) Size.

	Success Rate		
Perforation Size	Group I HAFGM	Group II Underlay	Group III Overlay
Small/grade I	94%	95%	94%
Medium/grade II	93%	92%	90%
Large/grade III	90%	89%	92%
Total/grade IV	88%	86%	89%
Global result	92.7%	92.2%	92.6%

HAFGM = hyaluronic acid fat graft myringoplasty.

Preoperative symptoms and previous surgeries were studied. Patients in group I noted a significantly greater history of otorrhea compared with group III (I vs. II, $P > .05$; I vs. III, $P = .005$) and tinnitus (I vs. II, $P = .01$; I vs. III, $P = .005$) compared with groups II and III. No differences were found for the preoperative hearing loss and otalgia symptoms. Patients in group I had more previous surgeries in the operated ear than patients in groups II ($P < .0001$) or III ($P < .0001$). Previous surgery included myringotomy tube (I: n = 28; II: n = 20; III: n = 16), myringoplasty and tympanoplasty (I: n = 17; II: n = 9; III: n = 10), and canal wall up mastoidectomy (I: n = 9; II: n = 0; III: n = 1). Previous surgery was not a factor affecting results of myringoplasty in any of the three groups ($P > .05$).

Perforations were classified into four grades based on the Saliba's classification reported in our previous publication:[12] grade I (small) for perforations less than 25% of the tympanic membrane surface (TMS); grade II (medium) for perforations between 25% and 50% of the TMS; grade III (large) for perforations between 50% and 75% of the TMS; and grade IV (total) for perforations more than 75% of the TMS. Groups II and III had more perforations of small and medium size than group I, which had more large and total perforations compared with groups II and III ($P = .012$) (Table II).

There was no statistically significant difference between group I and the other groups ($P > .05$) for the site of the perforation on the TM. The antero-superior quadrant was the least affected site in the three groups (Table II).

Global successful closure of the perforation was observed in 92.7% of group I patients without differences with group II (92.2%) or group III (92.6%). No difference of success was found between the three groups for each of the four grades of perforation. Detailed results are summarized in Table III.

Pre- and postoperative hearing test results are summarized in Table IV. No worsening of bone conduction threshold was noted postoperatively in the three groups. Thus HAFGM is a secure technique along with the conventional underlay and overlay techniques. Postoperative ABG was statistically similar for the three groups. Group I ABG improvement was clinically and statistically significant. Preoperative speech discrimination score (SDS) was 92.2%, 97.7%, and 89.28% for groups I, II, and III, respectively. Postoperatively, no clinically or statistically significant changes of the SDS were noted for the three

TABLE IV.
Preoperative and 12-Month Postoperative Mean Bone Conduction Threshold (BC) and Air Conduction Threshold (AC) in Decibel Hearing Level at Four Frequencies (± Standard Deviation).

		500 Hz	1,000 Hz	2,000 Hz	4,000 Hz
Group I HAFGM	Preoperative BC	17.8 ± 13.8	16.3 ± 15.3	22.6 ± 17.1	24.6 ± 21.7
	Postoperative BC	14.9 ± 12.7	14.6 ± 14.8	20.7 ± 17.4	25.5 ± 22.4
	Preoperative AC	44.9 ± 21.1	39.8 ± 19.4	39.6 ± 21.3	48.1 ± 26.7
	Postoperative AC	24.1 ± 15.9	24.7 ± 17.5	27.7 ± 18.8	39.4 ± 24.8
Group II Underlay	Preoperative BC	6.7 ± 9.8	6.7 ± 7.3	9.7 ± 10.4	5.9 ± 11.9
	Postoperative BC	9.1 ± 8.3	7.3 ± 6.8	9.5 ± 9.2	6.2 ± 12.1
	Preoperative AC	26.8 ± 15.2	21.7 ± 12.2	20.6 ± 14.8	21.8 ± 18.1
	Postoperative AC	21.7 ± 13.5	18.9 ± 12.3	17.5 ± 15.8	21.1 ± 16.9
Group III Overlay	Preoperative BC	10.3 ± 12.7	9.1 ± 12.5	12.5 ± 13.6	9.1 ± 15.1
	Postoperative BC	8.2 ± 11.7	9.4 ± 11.6	9.6 ± 12.7	7.6 ± 13.6
	Preoperative AC	33.9 ± 18.9	29.3 ± 19.1	25.2 ± 19.2	29.1 ± 23.1
	Postoperative AC	22.2 ± 16.2	21.5 ± 15.8	17.2 ± 16.4	25 ± 20.5

HAFGM = hyaluronic acid fat graft myringoplasty.

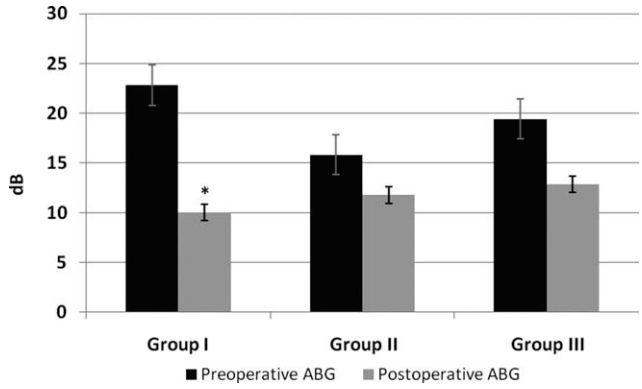

Fig. 1. Preoperative and 12-month postoperative air-bone gap (ABG) for the three groups (hyaluronic acid fat graft myringoplasty [HAFGM], group I; underlay technique, group II; overlay technique, group III). *Statistically significant difference ($P < .05$).

groups. The mean hearing improvement for the operated ears was 12.8 dB for group I, 3.69 dB for group II, and 6.3 dB for group III (Fig. 1).

The mean duration of the operative procedures including the anesthetic time was 16, 65, and 74 minutes for group I, II, and III, respectively ($P = .02$). All patients in group I were operated under local anesthesia in our otolaryngology outpatient department, whereas patients in groups II and III were operated under general anesthesia.

In group I, we estimated the fat graft lost to 50% of its bulging at the second postoperative month. At the fourth postoperative month, 20% to 30% of the original fat remained under the new epithelial cell sheath of the healed tympanic membrane. At 12 months postoperatively, we found a small stain of the fat graft in the tympanic membrane thickness. This remaining fat is the signature of HAFGM and persisted until the most recent follow-up for all patients in group I (maximum follow-up, 41 months).

The mean postoperative follow-up was 18.7, 20.6, and 15.5 months for groups I, II, and III, respectively. In unsuccessful cases, postoperative otitis was the cause of failure in 3.8% and 1.6% of patients in groups I and II, respectively ($P > .05$). No cause of failure was identified in group III. Residual perforation was identified in the first four postoperative months in 96.5% of failure cases for the three groups.

Minor complications were noted in the three groups: one case of tympanic pearl cholesteatoma was noted in group I and another in group III. One case of blunting was identified in group III. A retraction pocket was noted in two and four cases in groups I and II, respectively.

DISCUSSION

Ringenberg first described fat plug myringoplasty in 1962, with a success rate of 87%.[5] Since then, studies have shown success rates of FGM ranging from 76% to 92%.[7–9] All patients in these series, however, had small perforations. Dursun et al. recommended paper patch and fat myringoplasty for 1-mm and 2-mm perforations and perichondrium myringoplasty for 3-mm perforations via a transcanal approach under local anesthesia before referring a patient for classic myringoplasty.[13] Deddens et al. considered TM perforation size to be a crucial factor, thus perforations, in his series, were 5% to 30% of the drum surface, which he considered to be a good prognostic factor for a fat graft.[14] FGM successful closure was common in small tympanic membrane perforations, whereas graft failure rates were higher for perforations exceeding 50% of the pars tensa.[15] By adding HA to FGM, we observe in our series a high success rate independent of the perforation size and comparable to the underlay and overlay techniques.

The TM has a prominent autoreparative capacity. Unlike typical wound healing in other tissues, TM does not have a pre-epithelialization reaction of fibrous tissue ingrowth. There is a continuous centrifugal migration of the outer epithelial layer without a supportive matrix, thus preventing the influx of reparative cells and nutrients into the area of healing.[16] HA epidisc is believed to play a role in the healing regulation pattern of the fibrous layer, preventing dehydration of the perforation margins.[1,12,16] It also stimulates epithelial cells, accelerating centripetal migration of the epithelial layer over the temporary support of fat in the HAFGM technique.[12]

Stenfors concluded in his study that hyaluronic acid treatment may be an alternative to myringoplasty when the TM perforation is dry and less than one quadrant in size.[10] But the application was repeated every second to third day as long as there was a visible reduction in perforation size. The total number of HA applications in his study varied from 3 to 10, which is inconvenient and time-consuming for the patient and physician. The application of an HA epidisc that we used in the HAFGM technique was done once, at the same time as the myringoplasty. Patients were seen two months after the procedure, at which time the HA epidisc was completely dissolved in 80% of patients.

The success rate of overlay technique tympanoplasty varies from 91% to 97%. Underlay graft success rate ranges from 88% to 91%. This number climbs to 90% to 94% with the over-under tympanoplasty technique.[6] The global success rate in our series of HAFGM is 92.7%. It resembles the results of underlay and overlay myringoplasty, even though group I had a higher proportion of large and total perforation than groups II and III (Fig. 2).

HAFGM may overcome some healing difficulty of anterior perforations and the drawbacks of revision myringoplasty for both biological reasons,[1,3,17–21] by stimulating restoration of the fibrous layer and promoting revascularization, and for mechanical reasons,[8,11,17,20,22] where HAFGM plugging does not require exposure of the anterior middle ear, which is inadequate in the majority of cases, nor does it require any support at the level of the anterior annulus where the graft may lose the TM contact with traditional underlay myringoplasty. The anterior wall bulging of the external auditory canal should be drilled to expose the unidentified anterior rim of the perforation. HAFGM is limited by this inconvenience because

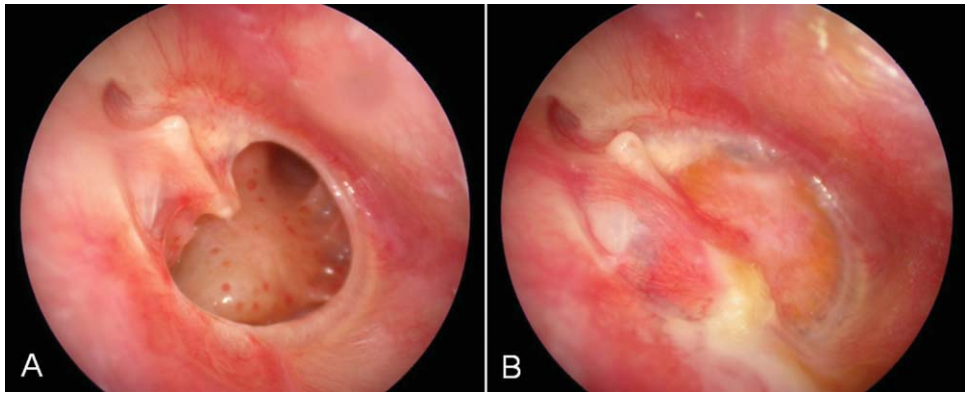

Fig. 2. (A) Hyaluronic acid fat graft myringoplasty (HAFGM) of a grade III right ear tympanic membrane perforation (TMP). (B) Complete closure of this perforation at four months postoperatively. The epithelium covering the fat graft remnant and the neovascularization can be identified.

it is performed in the clinic of the outpatient department. These cases were operated in the operating room by an overlay technique.

In TM perforations, the outer squamous epithelial layer grows medially, reaching the inner mucosal layer to form a contact inhibition zone, which denotes the persistence of a stable chronic perforation.[16] Posttraumatic perforation provides immediate exudation of tissue fluid, lymph, and/or blood at the margin, which stimulates the healing process.[23] A 6-month follow-up after a traumatic TM perforation is mandatory before a surgical decision is made. Like other techniques of myringoplasty, the first surgical step in HAFGM is to excise the rim of the perforation to remove epithelial migrating cells medially to the TM mucosa to stimulate the epithelial layer and to prevent cholesteatoma development. Complications of HAFGM are minimal. Small epithelial pearl on the TM or "drum cholesteatoma" is 50% less frequent than what it is reported for the overlay technique.[24] To prevent drum cholesteatoma in HAFGM, we should pay specific attention to cleaning the perforation rim from any medial epithelial migration. TM blunting or lateralization did not occur in any case of our HAFGM series.

Histologic properties of FGM have been investigated in guinea pigs and cats showing a normal-appearing outer epithelium and middle ear mucosa around a thick bulge of fat cells, and a variable amount of fibrous tissue.[22,25,26] Acute necrosis in the first days of implantation may explain initial reduction in volume of the graft, whereas apoptosis induced by cytokines and tumor necrosis factors, followed by macrophage removal of dead cells, account for later progressive reduction;[27] it

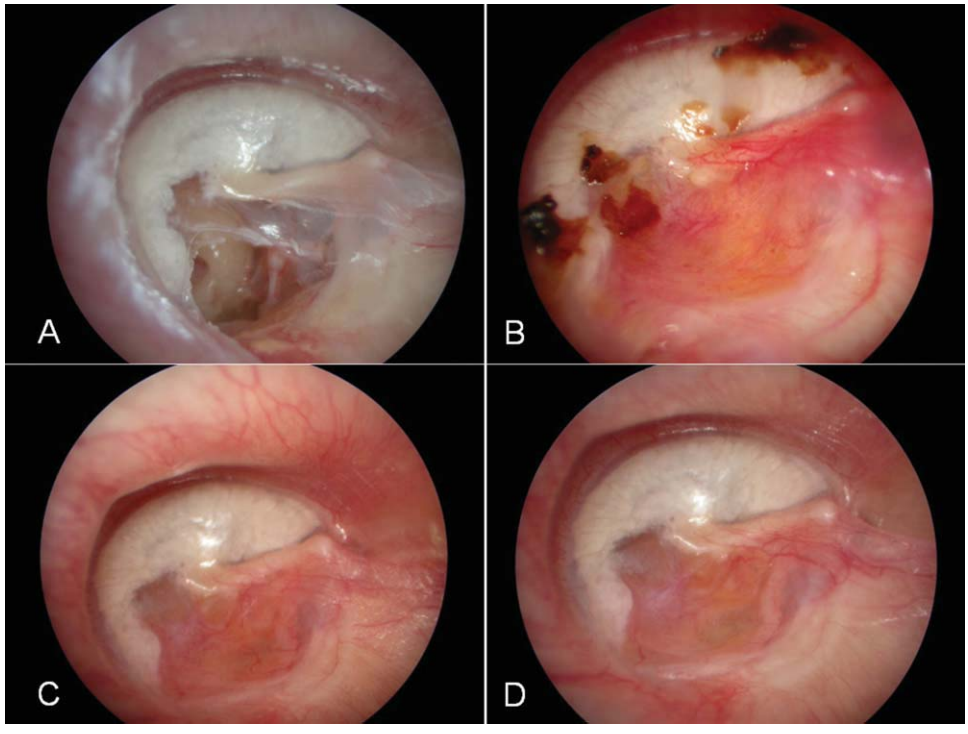

Fig. 3. Evolution of the fat graft throughout one year after a hyaluronic acid fat graft myringoplasty (HAFGM) for a left tympanic membrane perforation (TMP). (A) Preoperative TMP; (B) at two months postoperatively; (C) at 4 months postoperatively; and (D) at one year postoperatively.

is one of the reasons that we recommend harvesting a fat graft twice the size of the perforation. In our series, the fat graft lost its bulging on the tympanic membrane at the end of the second postoperative month. The fat graft progressively disappeared, leaving in its place at 12 months a thin sheath of fat between the new mucosa and the epithelial tissue (Fig. 3) without a sclerotic area on the tympanic membrane as reported by Ozgursoy.[3] HA may play a role in fat graft molding. Peer had also observed that transplanted fat cells were not replaced by scar tissue.[28] Our observation resembles that of Peer; at the first postoperative year, the operated tympanic membrane does not show any new sclerotic area but rather a normal TM appearance with a small, thin fatty stain in the epithelial thickness.

In our series, when graft failure occurred it was observed in the first four months after the HAFGM in the majority of cases. We did not observe late graft failure after the end of the fourth postoperative month with a maximum follow-up of 41 months. Success observed at the end of the fourth month persisted until the most recent appointment.

Patients in groups II and III were younger than group I patients. The effect of age is evident on the preoperative BC threshold and on tinnitus, which were markedly higher in the HAFGM group. In addition, the average perforation was larger in this group; therefore, the preoperative ABG was superior to groups II and III, giving a better ABG improvement. Nevertheless, postoperative ABG closure was similar for the three groups. HA is a non-ototoxic material.[29] Sensorineural hearing loss reported in patients who had undergone tympanoplasty procedures has not been noticed in any study on fat graft myringoplasty including our series.[5,8,14,30–32] HAFGM does not involve manipulation of middle ear structures and, compared with traditional myringoplasty, carries a low risk of iatrogenic otologic trauma. Gelfoam inserted in the middle ear to support the fat graft does not appear to affect the result of conductive hearing loss at the fourth postoperative month. Hearing tests at one postoperative year were similar to those done at four months. These pieces of gelfoam are important to prevent medialization of the fat graft. The comparative improvement of ABG for the three groups shows a high quality of TM recovery and serves as evidence that a small fatty thickness in the eardrum does not affect hearing result.

CONCLUSIONS

HAFGM is cost-effective and yields a higher success rate than reported results of FGM alone. Its success rate is comparable to that of underlay and overlay techniques, even in total perforation. Furthermore, it requires no hospitalization; it is performed as an office-based procedure, under local anesthesia for a mean operative time of 16 minutes. We avoid the potential complications and the difficulty of overlay and underlay tympanoplasty. For some patients, the risks, costs, and inconvenience of an operation are significant concerns. These patients may benefit from a simple, inexpensive, outpatient alternative and continue to work on the same day of the surgery.

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BIBLIOGRAPHY

1. Lim AA, Washington AP, Greinwald JH, Lassen LF, Holtel MR. Effect of pentoxifylline on the healing of guinea pig tympanic membrane. *Ann Otol Rhinol Laryngol* 2000;109:262–266.
2. Sckolnick JS, Mantle B, Li J, Chi DH. Pediatric myringoplasty: factors that affect success—a retrospective study. *Laryngoscope* 2008;118: 723–729.
3. Ozgursoy OB, Yorulmaz I. Fat graft myringoplasty: a cost-effective but underused procedure. *J Laryngol Otol* 2005;119:277–279.
4. Mudry A. History of tympanoplasty. *Otol Neurotol* 2005;26:554–555.
5. Ringenberg JC. Fat graft tympanoplasty. *Laryngoscope* 1962;72:188–192.
6. Yigit O, Alkan S, Topuz E, Uslu B, Unsal O, Dadas B. Short-term evaluation of over-under myringoplasty technique. *Eur Arch Otorhinolaryngol* 2005;262:400–403.
7. Landsberg R, Fishman G, DeRowe A, Berco E, Berger G. Fat graft myringoplasty: results of a long-term follow-up. *J Otolaryngol* 2006;35:44–47.
8. Terry RM, Bellini MJ, Clayton MI, Gandhi AG. Fat graft myringoplasty: a prospective trial. *Clin Otolaryngol Allied Sci* 1988;13:227–229.
9. Mitchell RB, Pereira KD, Lazar RH. Fat graft myringoplasty in children—a safe and successful day-stay procedure. *J Laryngol Otol* 1997;111: 106–108.
10. Stenfors LE. Repair of tympanic membrane perforations using hyaluronic acid: an alternative to myringoplasty. *J Laryngol Otol* 1989;103:39–40.
11. Prior M, Gibbins N, John G, Rhys-Williams S, Scott P. Hyaluronic acid ester in myringoplasty. *J Laryngol Otol* 2008;122:e3.Epub 2007 Dec 21.
12. Saliba I. Hyaluronic acid fat graft myringoplasty: how we do it. *Clin Otolaryngol* 2008;33:610–614.
13. Dursun E, Dogru S, Gungor A, Cincik H, Poyrazoglu E, Ozdemir T. Comparison of paper-patch, fat, and perichondrium myringoplasty in repair of small tympanic membrane perforations. *Otolaryngol Head Neck Surg* 2008;138:353–356.
14. Deddens AE, Muntz HR, Lusk RP. Adipose myringoplasty in children. *Laryngoscope* 1993;103:216–219.
15. Boyce RG, Nuss DW, Kluka EA. The use of autogenous fat, fascia, and non vascularized muscle grafts in the head and neck. *Otolaryngol Clin North Am* 1994;27:39–68.
16. Güneri EA, Tekin S, Yilmaz O, et al. The effects of hyaluronic acid, epidermal growth factor, and mitomycin in an experimental model of acute traumatic tympanic membrane perforation. *Otol Neurotol* 2003;24: 371–376.
17. Fiorino F, Barbieri F. Fat graft myringoplasty after unsuccessful tympanic membrane repair. *Eur Arch Otorhinolaryngol* 2007;264:1125–1128. Epub 2007 May 9.
18. Ayache S, Braccini F, Facon F, Thomassin JM. Adipose graft: an original option in myringoplasty. *Otol Neurotol* 2003;24:158–164.
19. Liew L, Daudia A, Narula AA. Synchronous fat plug myringoplasty and tympanostomy tube removal in the management of refractory otorrhoea in younger patients. *Int J Pediatr Otorhinolaryngol* 2002;66:291–296.
20. Imamoğlu M, Isik AU, Acuner O, Harova G, Bahadir O. Fat-plug and paper-patch myringoplasty in rats. *J Otolaryngol* 1998;27:318–321.
21. François M, Juvanon JM, Contencin P, Bobin S, Manac'h Y, Narcy P. Myringoplasty in children. *Ann Otolaryngol Chir Cervicofac* 1985;102: 321–327.
22. Reijnen CJH, Kuijpers W. The healing pattern of the drum membrane. *Acta Otolaryngol (Stockh)* 1971;287(suppl):1–74.
23. Stenfors LE, Salen B, Winblad B. The healing pattern of experimental tympanic membrane perforations. *Acta Otolaryngologica* 1980;90: 267–274.
24. El-Seifi A, Fouad B.The fibrous annulus in myringoplasty. *J Laryngol Otol* 1992;106:116–119.
25. Gold SR, Chaffoo RA. Fat myringoplasty in the guinea pig. *Laryngoscope* 1991;101(Pt 1):1–5.
26. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13:4279–4295.
27. Nishimura T, Hashimoto H, Nakanishi I, Furukawa M. Microvascular angiogenesis and apoptosis in the survival of free fat grafts. *Laryngoscope* 2000;110:1333–1338.
28. Peer LA. *Transplantation of tissues, vol 2: Transplantation of fat*. Baltimore: Williams & Wilkins; 1959.
29. Li G, Feghali JG, Dinces E, McElveen J, van de Water TR. Evaluation of esterified hyaluronic acid as middle ear-packing material. *Arch Otolaryngol Head Neck Surg* 2001;127:534–539.
30. Mitchell RB, Pereira KD, Younis RT, Lazar RH. Bilateral fat graft myringoplasty in children. *Ear Nose Throat J* 1996;75:652.655–656.
31. Laurent C, Söderberg O, Anniko M, Hartwig S. Repair of chronic tympanic membrane perforations using applications of hyaluronan or rice paper prostheses. *ORL J Otorhinolaryngol Relat Spec* 1991;53:37–40.
32. Goodman WS. Tympanoplasty: areolar tissue graft. *Laryngoscope* 1971;81: 1819–25.